Assessing the Diversity of *Pythium* species and Fungicide Efficacy in Agronomic Production Fields in Ohio

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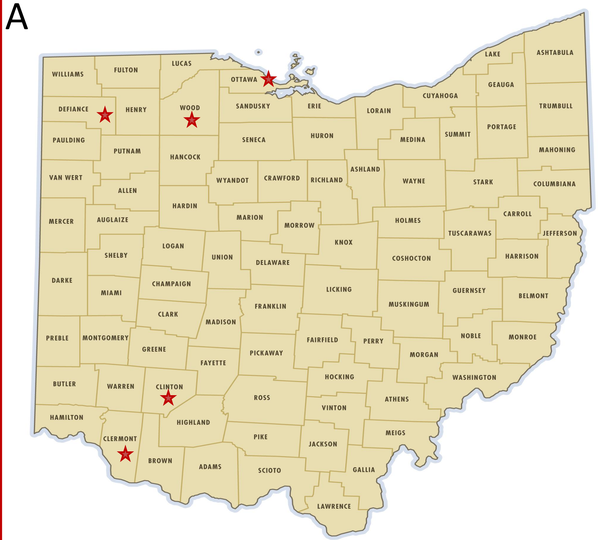
Abstract

The occurrence of seedling diseases in soybean fields in Ohio has increased over the past decade. There are at least 22 possible species of *Pythium* that are pathogenic to soybean. During the spring of 2008, field plots were planted at five different locations across the soybean production regions in Ohio, in a randomized block design with eight different fungicide seed treatments and four replications. The fungicide seed treatments included; mefenoxam, fludioxonil, azoxystrobin, mefenoxam + fludioxonil, mefenoxam + azoxystrobin, fludioxonil + azoxystrobin, and mefenoxam + fludioxonil + azoxystrobin. Stand counts at R1 and yield were recorded. *Pythium* species were isolated from symptomatic plants at these locations and the isolates were then identified to species using single strand conformation polymorphism. There were no significant interactions between location and seed treatment for stand count. Stands from seed treated with azoxystrobin, mefenoxam + fludioxonil, fludioxonil + azoxystrobin, and mefenoxam + fludioxonil + azoxystrobin treatments were significantly higher than the untreated check for stand count, but not for yield. A total of 13 *Pythium* species were recovered from symptomatic plants from the five locations. *Pythium irregulare*, *P. dissotocum*, and *P. torulosum* were the most frequently recovered species from all five locations. The results from this study will be used for the development of disease management strategies for seedling pathogens in Ohio.

Introduction

Numerous *Pythium* spp. contribute to the soybean seedling disease complex in Ohio (Dorrance et al. 2004, and Broders et al. 2007). Previous surveys recovered 22 different species of *Pythium* in Ohio where stand establishment problems were prevalent for soybean (Broders et al., 2007; Broders et al. 2008). Most seedling diseases, including early season damping-off, are managed by planting seed treated with a combination of seed-applied fungicides. Broders et al. (2007) evaluated the sensitivity of 13 *Pythium* spp. in vitro to four commercial seed treatment fungicides, finding none provided control for all 13 species, and there was a significant amount of variation in sensitivity to the fungicides among isolates both across species and within a species. In Ohio, a particular fungicide may perform well and decrease disease in one field but may perform poorly having little or no effect in another field. This may be due in large part to the *Pythium* community present in that particular field. Therefore the objectives of this study were: i) to identify the *Pythium* species present in five different locations isolating directly from diseased plants collected in the fields, ii) and to evaluate seed treatment fungicides, in on-farm studies for the efficacy against previously characterized *Pythium* communities.

A



B

C

Border range

4 row border	401 3	402 6	403 5	404 2	405 1	406 8	407 7	408 4	4 row border
	301 1	302 5	303 2	304 6	305 3	306 8	307 4	308 7	
	201 5	202 1	203 3	204 6	205 2	206 8	207 4	208 7	
	101 1	102 2	103 3	104 4	105 5	106 6	107 7	108 8	

Border range

Figure 1. A) The study was located in five fields in Ohio, indicated by stars. B) Soybean trial at the Clinton location. C) Experimental design used at four of the five locations.

Material and Methods

- During the spring of 2008, field plots were planted in Clermont, Clinton, Defiance, Ottawa, and Wood County (Fig. 1a). The experimental design was a randomized block design with eight different fungicide treatments (Table 1, Fig. 1c).
- Soybean seed was provided and treated by Syngenta seeds.
- Stand counts were recorded at R1 at each of the five locations. All plants were counted in the two center rows. During this time symptomatic plants were collected for isolation of *Pythium* spp. and the isolates were then identified using single strand conformation polymorphism (SSCP) (Fig. 2).
- Yield data was recorded at the end of the growing season.
- Percent stand count and yield data were analyzed using PROC GLM of SAS (SAS Institute Inc., Cary, NC).

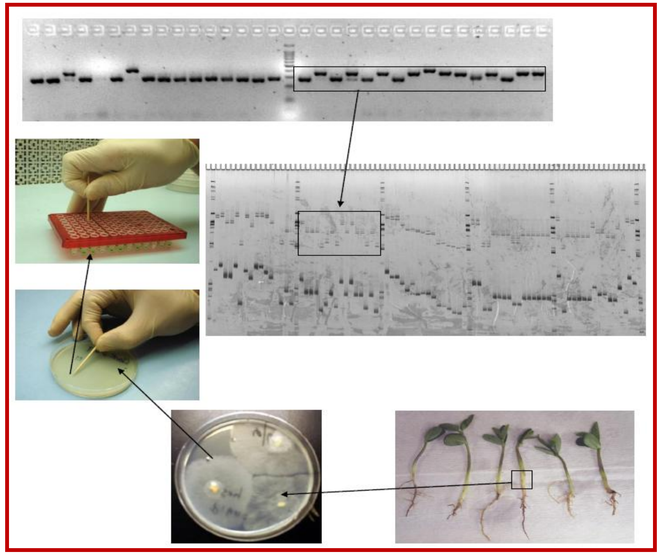


Figure 2. Symptomatic plants were collected from the field and brought back to the lab. The infected plant tissue was plated on PIBNC, an Oomycete specific media. Pythium isolates were then transferred to potato-carrot agar. Product from direct colony PCR, using the ITS 6 and ITS 7 primers, was used for SSCP.

Table 1: Fungicide Seed Treatments

Seed treatment	Trade Names
Mefenoxam	Apron XL 3 LS
Fludioxonil	Maxim 4 FS
Azoxystrobin	Dynasty .83 FS
Mefenoxam + Fludioxonil	Apron XL 3 LS + Maxim 4 FS
Mefenoxam + Azoxystrobin	Apron XL 3 LS + Dynasty .83 FS
Fludioxonil + Azoxystrobin	Maxim 4 FS + Dynasty .83 FS
Mefenoxam + Fludioxonil + Azoxystrobin	Apron XL 3 LS + Maxim 4 FS + Dynasty .83 FS

Table 1. Products registered by Syngenta Crop Protection, Inc.

Literature

Broders, K. D. 2008. Seed and seedling disease of corn and soybean in Ohio: The role of *Fusarium graminearum, Pythium species diversity, fungicide sensitivity, Pythium* community composition, and soil properties on disease severity. Ph.D. Dissertation, The Ohio State University 204 pp.

Broders, K. D., Lipps, P. E., Paul, P. A., and Dorrance, A. E. 2007. Characterization of *Pythium* spp. associated with corn and soybean seed and seedling disease in Ohio. Plant Dis. 91:1155-1160.

Dorrance, A. E., Berry, S. A., Bowen, P., and Lipps, P. E. 2004. Characterization of *Pythium* spp. from three Ohio fields for pathogenicity on corn and soybean and metalaxyl sensitivity. Plant Heath Progress. Plant Management Network. Online publication.

Acknowledgement

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Results

- There was no significant interaction between location and seed treatment ($P = 0.2875$) for percent stand.
- Seed treatments, azoxystrobin, mefenoxam + fludioxonil, fludioxonil + azoxystrobin, and mefenoxam + fludioxonil + azoxystrobin were significantly higher than the untreated check ($P = 0.05$) (Table 2).
- There was no significant difference for yield for the seed treatments.
- Of the 13 species of Pythium recovered from symptomatic plants. *Pythium dissotocum, P. torulosum, and P. irregulare* were the most frequently recovered species from all five locations. *Pythium ultimum* var. *ultimum*, *P. ultimum* var. *sporangiiferum*, *P. inflatum*, were the next most frequent species recovered from three locations (Table 3).

Table 2: Percent stand results comparing fungicide seed treatments in five fields in Ohio

Treatment	Percent Stand
Mefenoxam	71.9ab
Fludioxonil	66.9c
Azoxystrobin	74.1a
Mefenoxam + Fludioxonil	73.4a
Mefenoxam + Azoxystrobin	71.3ab
Fludioxonil + Azoxystrobin	73.2a
Mefenoxam + Fludioxonil + Azoxystrobin	73.3a
Untreated check	67.8bc
Mean	71.5

Table 3: Species of *Pythium* recovered from five fields in Ohio

	Counties					
Species	Clermont	Clinton	Defiance	Ottawa	Wood	Total number of isolates identified
P. attrantheridium				4	1	5
P. dissotocum	2	9	1	36	2	50
P. heterothallicum		1				1
P. inflatum	1	6		2		9
P. irregulare	5	4	1	11	2	23
P. longandrum				1		1
P. pleroticum	2			1		3
P. rostratum			1			1
P. sylvaticum	9					9
P. torulosum	4	6	4	8	2	24
P. ultimum var. sporangiferum		6		4	2	12
P. ultimum var. ultimum	6	14	1			21
G7		2			1	3
Total number	29	48	8	67	10	162

Table 2. Values in a column followed by the same letter are not significantly different according to Fisher's protected least significant difference ($P < 0.05$). The experiment was a randomized block design with four replications.

Discussion

The results from this research correspond to previous survey work done by Broders et al. 2007, 2008. Ottawa and Clinton counties had eight species compared to seven, six, and five for Clermont, Wood, and Defiance counties, respectively. Ottawa and Clinton counties had the greatest number of isolates recovered from soybean. This study will be repeated during 2009 and the results from the study will assist growers in management strategies for the *Pythium* disease complex in Ohio Soybean fields.